Response of *Aspergillus* species to the Toxicity of Domestic Detergents

Williams, Janet Olufunmilayo and Ogolo, Chinomso Nwaka
Department of Microbiology, Rivers State University, Port Harcourt, Rivers State, Nigeria.
08023382630

Abstract: This study was performed to assess the toxicity of two different domestic detergents at varying concentrations on the *Aspergillus* sp. subjected to different exposure times. The toxicological assessment was performed using the *Aspergillus species* isolated from fresh (Asarama stream, Asarama town, Andoni L.G.A) and brackish (Eagle island River, Port Harcourt) aquatic ecosystems both in Rivers state. The set-ups containing the test microorganism had the following concentrations of domestic detergents (Mymy and Zip): 6.25%, 12.5%, 25%, 50% and 75% and were exposed for 0, 4, 8, 12 and 24hrsrespectively.With the use of differential/selective media, the organism was isolated and further characterized by cell colonial and morphological identification. The median lethal concentration (Lc_{50}) was employed to compute the toxicities of the two domestic detergents on the test organism. The result obtained showed that the *Aspergillus* sp. demonstrated great sensitivity to the toxicity elicited by the domestic detergents. The sensitivity of the *Aspergillus* sp. to the toxicity of the different toxicants, Mymy and Zip detergents from the two different water samples decreased in the following order (noting the lower the lethal concentration (Lc_{50}), the more toxic the toxicant): Zip detergent in freshwater (37.26 mg/I) >Mymy detergent in brackish water (41.71 mg/1) > Zip detergent in brackish water (42.63 mg/1) >Mymy detergent in fresh water (47.82 mg/1). The Zip detergent showed more toxicity in fresh water with Lc_{50} of 37.26 and Mymy detergent showed less toxicity in fresh water with Lc_{50} of 47.82.

Keywords: *Aspergillus* species, toxicity, Mymy, zip, detergents, fresh and backish water.

Introduction

Detergents, either domestic or industrial, have several useful applications in our society. Hence, they are used as a means of generating income. It can be used domestically for cleaning, medically for sterilization and industrially for the same or different purposes. In Nigeria, surfactant detergents are in widespread usage and are regularly used in several domestic washing activities. However, the discharge of waste containing domestic or industrial detergents into the ecosystem affects the macro and micro biota of the receiving environment. Detergents have poisonous effects on all types of aquatic life when they are present in sufficient amount. While in soil, they act as a major agent of the reduction of water hydraulic conductivity of soils with adverse impacts on agricultural productivity and environmental sustainability (Odokuma and Akponah, 2008).

Generally, detergents have effects that can be severe on the health and growth of wild life, humans and microorganisms due to their toxicological properties (Birkett and Lester, 2003). An anionic surfactant most extensively used as a key ingredient in domestic and industrial detergents is linear alkylbenzenesulphonate (LAS) which can be simply degraded in waste water treatment plants by microorganisms using aerobic processes. However, the presence of LAS may retard, inhibit or reduce the population of the microflora in an ecosystem by inhibiting their proliferation as a result of its toxicity which can be lethal or inhibitory (Leon *et al*., 2006).Detergents are organic compounds which possess both polar and non-polar characteristics. They are of three types; anionic, cationic and non-ionic. The anionic and cationic types are said to have permanent charges of either negative or positive charge attached to non-polar (hydrophobic) C-C chains. The non-ionic detergent does not have these permanent charges. Rather, they possess atoms which can either be weakly electronegative or electropositive (Obire and Nrior, 2014). This is as a result of electron affecting strength of the oxygen atoms. Detergents are further subdivided into two sub-groups with different characteristics; phosphate detergents and surfactant detergents. Phosphate detergents are highly caustic and surfactant detergents are highly toxic (Lenntech, 2008). Surfactant detergents are used widely in Nigeria both domestically and industrially.

Surfactants in detergents are released into the environment via the discharge of untreated waste water by industries, companies or homes. In addition to these surfactants, the discharge of untreated waste water into the soil may contain certain elements (for example, iron, lead, phosphorous, calcium and zinc) previously absent or present in minute quantities which will be introduced into the environment leading to magnification of these chemicals, thus, altering the physiochemical nature of the soil. Some of these chemicals may be toxic to the microbial flora and fauna communities of the soil (Williams and Dimbu, 2015).

*Corresponding author:
*janet.williams@ust.edu.ng * Williams, Janet lufunmilayo*

Detergents have toxic effect on all types of aquatic life when they are present in high quantities and this includes the biodegradable detergents. All detergents destroy the external mucus layer that protects the fish from bacteria and parasites plus they can cause severe damage to gills and most fishes will die when detergent concentrations approach 15 parts per million (ppm) (Obire and Nrior, 2014).

Aspergillus sp. is one of the most important microorganisms used in biotechnology which produces many extracellular enzymes (Stojanovic *et al.*, 2011). *Aspergillus* species are well-known producers of a wide spectrum of mycotoxins including aflatoxins, sterigmatocystin and Ochratoxins which are causative agents of numerous carcinogenic, hepatogenic and nephrogenic ailments as well as producing immuno suppressive effects. However, most of the members are useful microorganisms in nature for the degradation of plant polysaccharides (De Vries, 2003) and they are also important microorganisms for the large-scale production of both homologous and heterologous enzymes (Fawole and Odunfa, 2003). *Aspergillus niger* is one of the most important microorganisms used in biotechnology which produces many extracellular enzymes (Contesini *et al.*, 2010). *Aspergillus* sp. has also been discovered to play a vital role in nitrogen fixation.

Toxicity is an evaluation of how toxic (poisonous) a substance is (Clark, 1992). It is the potential or ability of a test material (detergent) to cause harm on living organisms due to its unfavorable effect. Although, the concept of toxicity seems straight forward, it appears to have some complicating factors during measurement. A commonly used measure of acute toxicity is the 50% lethal Concentration (LC_{50}) or dose (LD_{50}) which is the concentration of the toxicant at which 50% of the population of organism is killed.

Historically and up to date, conventional animals such as rats, rabbits and mice were employed for the assessment of the toxicity of chemicals. Other macro organisms such as fish, crab, snail and crayfish have also been used for toxicity bioassay (Atuanyan and Tudararo-Aherobo, 2015). Use of these macro organisms in the eco-toxicological assessment consumes time and cost of operation is also high. These species present various problems that influence their choice as test organisms. This problem includes high death rate as a result of stress, inadequacies in feeding and the inability to adjust to experimental environment. However, the ideal characteristics for a bioassay testing organism were given by Williamson and Johnson in 1981 (Williamson and Johnson, 1981). This includes its sensitivity and convenience, genetic stability, consistency of organism's response to toxicants, etc. In all of these, one can possibly say that microorganisms can effectively fit in as excellent bioassay tools for eco-toxicological assessment as they are meant to meet up with the increasing demand of rapid, inexpensive and relatively simple screening tests for eco-toxicological evaluation (Odokuma and Okpokwasili, 2003).

With regards to this study, the fungus,*Aspergillus sp.*is used as the bioassay tool for the assessment of the short-term toxicity of two (2) synthetic and industrially produced domestic detergents (Mymy and Zip).The aim of this study was to assess the toxicity of some domestic detergents (Mymy and Zip) with different concentrations on the growth of *Aspergillus* sp. Isolated from two aquatic environments (fresh and brackish) as an eco-toxicological tool with respect to other forms of life.

Materials and Methods

Sample Collection/Study Area

Brackish water sample was collected from Eagle Island River, Port Harcourt in Rivers State while fresh water sample was collected from Asarama stream, Asarama Town, Andoni L.G.A, Rivers State. Both samples were collected in 100ml sterile plastic containers, taken to the Microbiology Laboratory, Rivers State University in ic fixed coolers and used for the analysis within two (2) hours of collection for the isolation of *Aspergillus* spp

Isolation of Test Organism

Aspergillus species in this study were isolated from the water samples collected from Asarama stream and Eagle Island River. About one milliliter (1ml) each of the water samples were measured and aseptically transferred into 9ml of sterile physiological saline contained in test tubes plugged with cotton wool. This was properly shaken and serial dilution was done up to the third dilution factor (10^{-3}). Then 0.1ml each of the suspensions was collected using sterile glass pipettes and aseptically inoculated into sabouraud dextrose agar using the spread plate method for the isolation of some species of *Aspergillus*. Incubation of all plates followed immediately at a room temperature of 28◦c for 3-5 days. The colonies that developed were sub-cultured and identified based on morphological characteristics.

Aspergillus niger isolated from the fresh water (Asarama river) was sub cultured in Sabouraud dextrose broth at temperature of 28^{0}C for 5days and was used for the toxicity test.

Source of Detergents (toxicants)

The domestic detergents used in this study were powdered detergents (Mymy and Zip). Mymy detergent is produced by Daraju Industries Limited, Ogun State and Zip detergent is produced by PZ Cussons Nigeria plc, Lagos State, Nigeria but they were obtained from Mile 3 market, Diobu in Port Harcourt.

Microbiological Analyses of Water Samples

Enumeration and Identification of fungal species

Water samples were used for the enumeration of fungal species. Samples were serially diluted and an aliquot from each sample was placed on acidified

potato dextrose agar plates containing streptomycin (1mg/100ml) for isolation of fungal isolates. The plates were incubated at 30°C and observed after 96 hours for the mould, after this, isolation of pure isolate was done (Williams and Money, 2017, Williams and Madise,2018).

Toxicity Test

Preparation of Toxicants

The toxicants were prepared by setting up 2 sets of test tubes with each set having 6 test tubes respectively making a sum of 12 test tubes. The twelve test tubes were containing appropriate filtered water (fresh and brackish water from the habitat of the organism) with all test tubes aseptically covered with cotton wool. In each set of test tubes containing either fresh or brackish water, the five toxicant concentrations (6.25%, 12.5%, 25%, 50% and 75%) were added separately having one control from each set of test tubes with no toxicant.

Test Procedures for Fresh and Brackish Water containing toxicants and *Aspergillus* species

About 1ml of the test organism from the sabouraud dextrose broth was added to separate toxicant concentrations (6.25%, 12.5%, 25%, 50% and 75%) and the controls in the two sets of test tubes respectively and an aliquot (0.1ml) was immediately plated out after inoculation on the sabouraud dextrose agar plates. This is known as the zero (0) hour count plating and was incubated at a temperature of 37^0C. Subsequently, an aliquot from each concentration of the effluent was then plated after 4hours, 8hours, 12hours and 24hours respectively on sabouraud dextrose agar and was incubated for 48 hours. Colonies observed were counted and recorded.

Percentage (%) Log Survival of *Aspergillus* species with the Domestic Detergents

The percentage log survival of the isolate (*Aspergillus* sp.) with the domestic detergents used in the study was calculated using the formula adopted from Williamson and Johnson (1981). The percentage log survival of the fungal isolate in the effluent was calculated by obtaining the log of the count in toxicant concentration, dividing it by the log of the count in the zero toxicant concentration and multiplying by 100. Thus,

$$\text{Percentage (\%) log survival} = \frac{\text{Log C x 100}}{\text{Log c}}$$

Where:
LogC = log of the count in each toxicant concentration
Logc = log of the count in the control (zero toxicant concentration)

Percentage (%) Mortality of Fungus in Fresh and Brackish water containing toxicants

This study was carried out to assess the probable toxic effect domestic detergents (Mymy and Zip) could have in fresh and brackish water (aquatic environment). The formula for the calculation of percentage mortality was adopted from APHA (1992). This is done by subtracting percentage (%) log survival from the percentage (%) log control. This is shown with the formula below Percentage (%) mortality = zero toxicant concentration - % log survival.

Results

The toxicity test was carried out using *Aspergillus* species containing two toxicants (Mymy and Zip) in fresh and brackish water and the median lethal concentrations(Lc_{50}) of the toxicants on the organism was calculated. The lethal toxicity of the detergents on the organism was done by calculating the percentage (%) log survival which was obtained by dividing the log count of each toxicant concentration by the log control and multiplying it by 100 and further subtracting it from the percentage (%) log control to obtain the percentage (%) mortality.

Percentage (%) log survival of *Aspergillus* species in fresh and brackish water containing toxicants

Figures 1, 5 and 2, 6 below shows the percentage (%) log survival of *Aspergillus* sp. in fresh and brackish water respectively containing Mymy detergent, while fig. 3,7 and 4, 8 shows the percentage (%)log survival of *Aspergillus* sp. in fresh and brackish water respectively containing Zip detergent. Table 5 shows the summary of the median lethal concentration of Mymy and Zip detergents on *Aspergillus* sp. in fresh and brackish water.

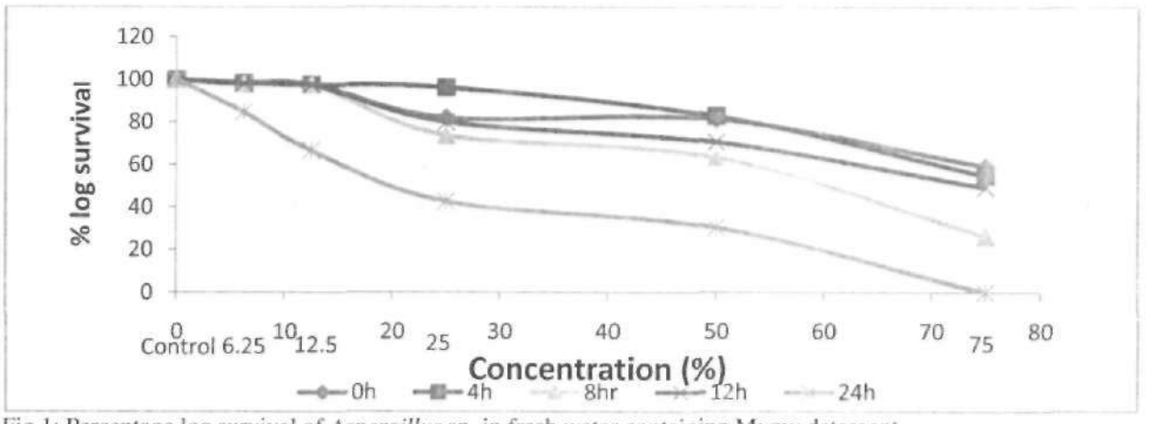

Fig.1: Percentage log survival of *Aspergillus* sp. in fresh water containing Mymy detergent

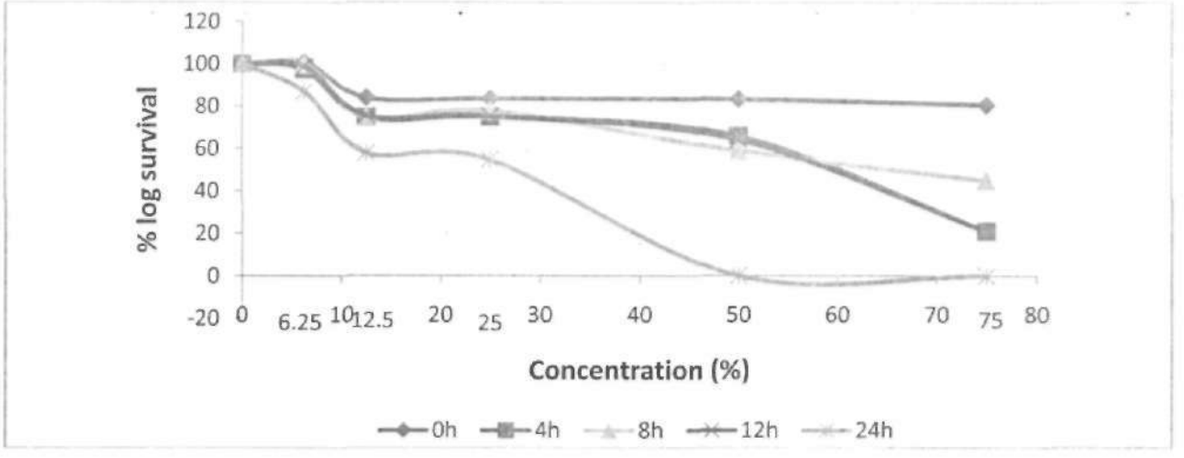

Fig. 2: Percentage (%) log survival of *Aspergillus* sp. in brackish water containing Mymy detergent

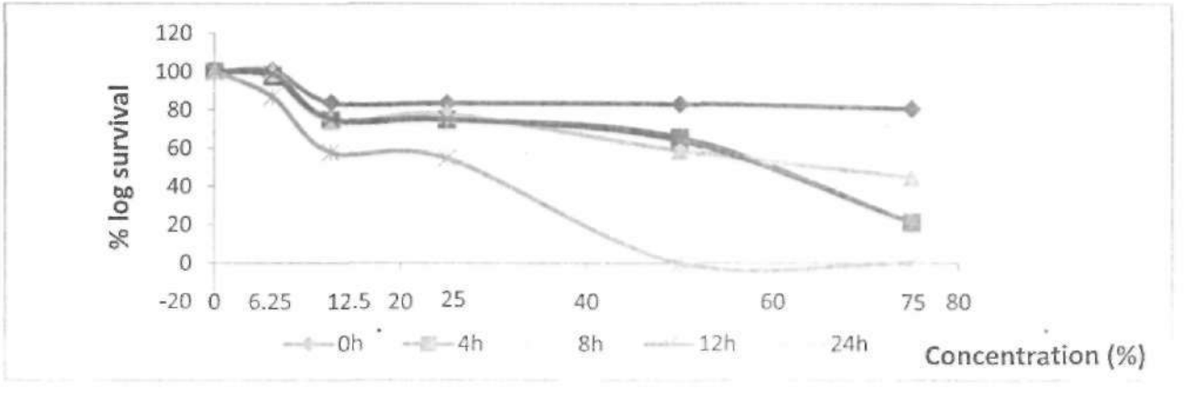

Fig.3: Percentage (%) log survival of *Aspergillus* sp. in fresh water containing Zip detergent.

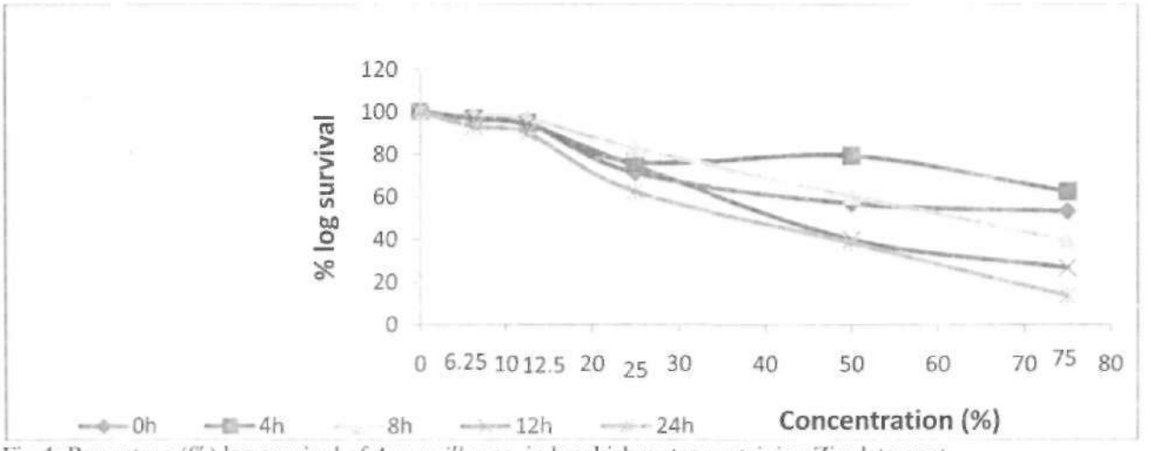

Fig.4: Percentage (%) log survival of *Aspergillus* sp. in brackish water containing Zip detergent

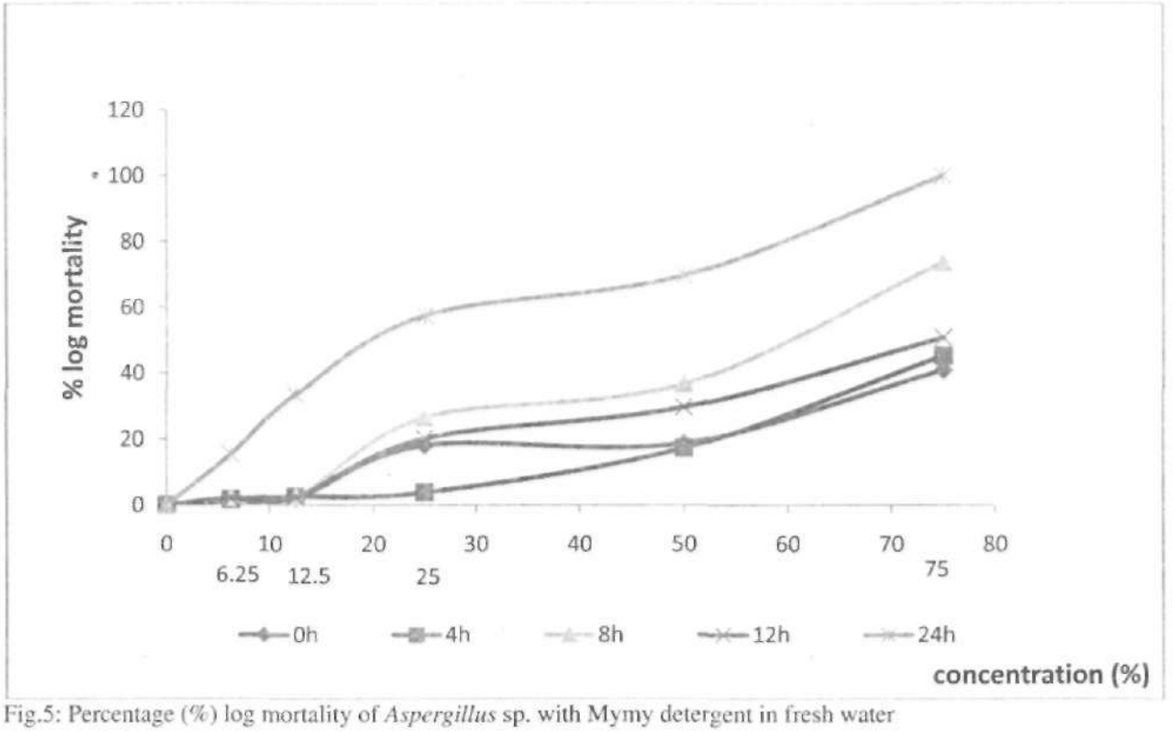

Fig.5: Percentage (%) log mortality of *Aspergillus* sp. with Mymy detergent in fresh water

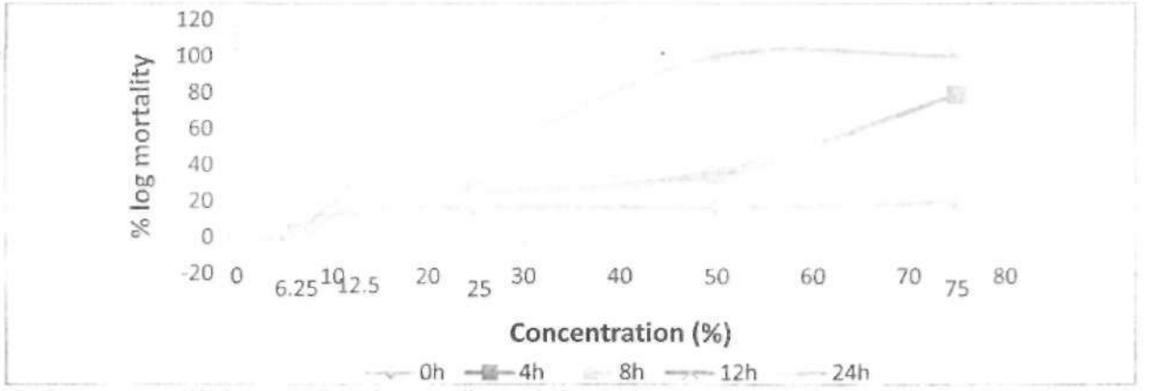

Fig.6: percentage (%) log mortality of *Aspergillus* sp. in brackish water containing Mymy detergent

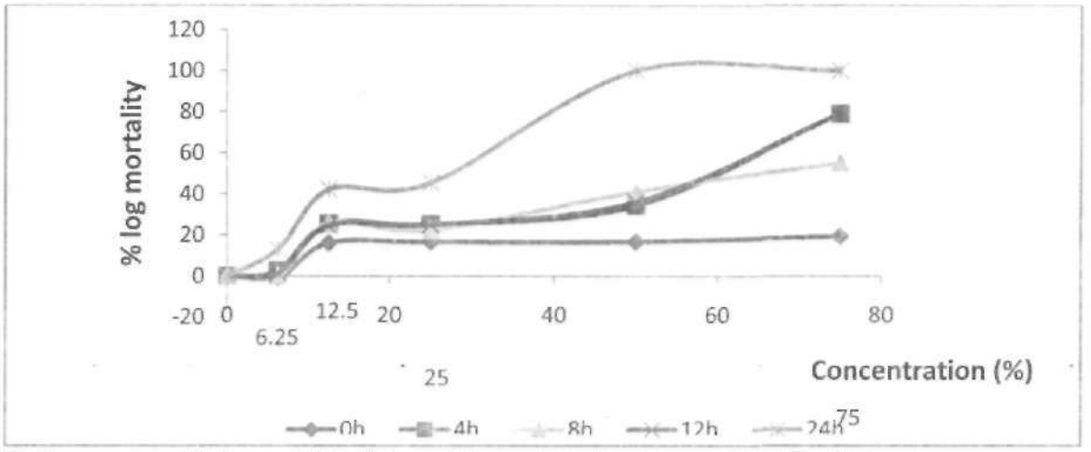

Fig.7: percentage (%) log mortality of *Aspergillus* sp. in fresh water containing Zip detergent

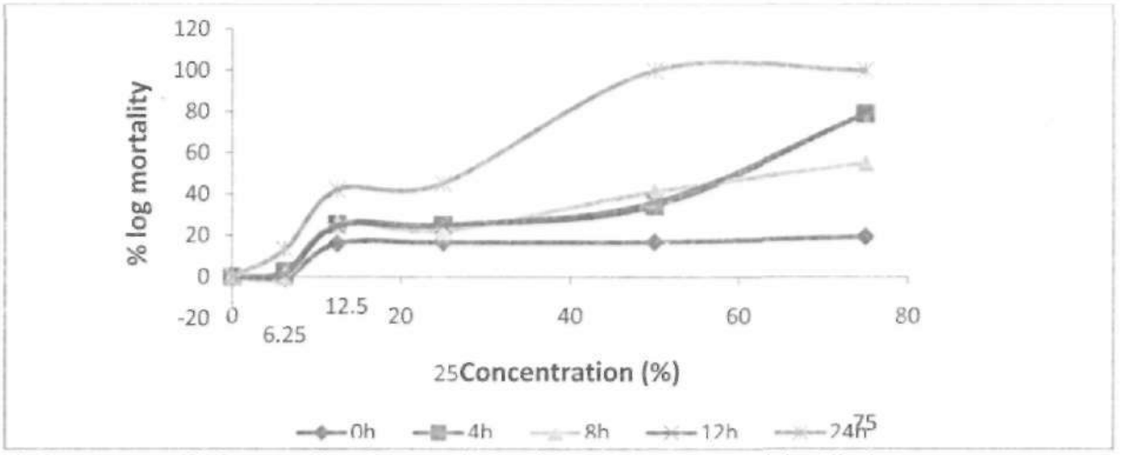

Fig.8: percentage (%) log mortality of *Aspergillus* sp. in brackish water containing Zip detergent

Median lethal concentration (Lc_{50}) of the domestic detergents on *Aspergillus* sp. in fresh and brackish water

Table 1 shows the median lethal concentration (Lc_{50}) of Mymy detergent on *Aspergillus* sp. in fresh water. This was obtained by subtracting the sum of the dose difference multiplied by mean percentage (%) mortality and divided by the percentage (%) control from the highest toxicant concentration as seen in the formula below derived by Williamson and Johnson.

$$Lc_{50} = \frac{Lc_{100} - \sum \text{dose diff.} \times \text{mean \% mortality}}{\text{\% Control}}$$

WHERE

Lc_{100} is the highest toxicant concentration

Table 1: Median lethal concentration (Lc_{50}) of Mymy detergent on *Aspergillus* sp. in fresh water

Dose (%)	% mortality	mean % mortality	dose diff.	Dose diff. × Mean% mortality
0	0	0	0	0
6.25	22.84	4.568	6.25	28.55
12.5	43.13	8.626	6.25	53.91
25	126.18	25.236	12.5	315.45

50	153.25	30.65	25	766.25
75	310.73	62.146	25	1,553.65
				2,717.81

$$Lc_{50} = \frac{Lc_{100} - \sum \text{dose diff.} \times \text{mean \% mortality}}{\text{\% Control}}$$

$$= \frac{75 - 2{,}717.81}{100}$$

$$= 75 - 27.18$$

$$= 47.82$$

Table 2 shows the median lethal concentration (Lc_{50}) of Mymy detergent on *Aspergillus* sp. in brackish water.

Table 2: Median lethal concentration (Lc_{50}) of Mymy detergent on *Aspergillus* sp. in brackish water

Dose (%)	% mortality	mean % mortality	dose diff.	Dose diff. × Mean% mortality
0	0	0	0	0
6.25	16.73	3.346	6.25	20.913
12.5	133.93	26.786	6.25	167.413
25	134.43	26.886	12.5	336.075
50	228.32	45.664	25	1,141.60
75	332.49	66.498	25	1,662.45
				3,328.451

$$Lc_{50} = \frac{Lc_{100} - \sum \text{dose diff.} \times \text{mean \% mortality}}{\text{\% Control}}$$

$$= \frac{75 - 3{,}328.451}{100}$$

$$= 75 - 32.29$$

$$= 42.71$$

Table 3 shows the median lethal concentration (Lc_{50}) of zip detergent on *Aspergillus* sp. in fresh water.

Table 3: Median lethal concentration (Lc_{50}) of Zipd etergent on *Aspergillus* sp. in fresh water

Dose (%)	% mortality	mean % mortality	dose diff.	Dose diff. ×Mean% mortality
0	0	0	0	0
6.25	22.24	4.448	6.25	27.8
12.5	106.83	21.366	6.25	133.54
25	177.23	35.446	12.5	443.08
50	291.39	58.278	25	1,456.95
75	342.61	68.522	25	1,713.05
				3,774.42

$$Lc_{50} = \frac{Lc_{100} - \sum \text{dose diff.} \times \text{mean \% mortality}}{\text{\% Control}}$$

$$= \frac{75 - 3{,}774.42}{100}$$

$$= 75 - 37.74$$

$$= 37.26$$

Table 4 shows the median lethal concentration (Lc_{50}) of zip detergent on *Aspergillus* sp. in brackish water.

Table 4:Median lethal concentration (Lc_{50}) of Zip detergent on *Aspergillus* sp. in brackish water

Dose (%)	% mortality	mean % mortality	dose diff.	Dose diff. × Mean% mortality
0	0	0	0	0
6.25	17.81	3.562	6.25	22.263
12.5	30.37	6.074	6.25	37.96
25	132.62	26.52	12.5	331.55
50	225.01	45.00	25	1,125.05
75	344.02	68.80	25	1,720.1
				3,236.92

$$Lc_{50} = Lc_{100} - \frac{\sum \text{dose diff.} \times \text{mean \% mortality}}{\text{\% Control}}$$

$$= \frac{75 - 3,236.92}{100}$$

$$= 75 - 32.37$$

$$= 42.63$$

Table 5: Summary of the median lethal concentration of Mymy and Zip detergents on *Aspergillus* sp. in fresh and brackish water.

	Fresh water	brackish water
Mymy detergent	47.82	41.71
Zip detergent	37.26	42.63

Discussion

The toxicity assess mentor bioassay was performed using *Aspergillus* species due to its simplicity, rapidity and low cost procedure as well as many other advantages. From the study, it was observed that the two domestic detergents employed in this study posed lethal to the biological population of the two aquatic environments and seriously altered the overall ability of the river ecosystem. The same was observed with the response of *Aspergillus niger* to the toxicity of detergent with respect to other forms of life (Obire and Nrior, 2014). However, the result of the percentage (%) log survival count showed that *Aspergillus* sp. is quite tolerant to the toxicants at some concentrations and exposure times as seen on figure 1. It showed that the organism had reasonable growth at 6.25% and 12.5% concentrations of the toxicants at 0, 4, and 8 and to some extent, 12 and 24 hours. The tolerance shown by *Aspergillus* sp. to the toxicants (domestic detergent) may be due to its ability to utilize some components of the detergent as carbon source (Focht and Weslake, 1987) or develop a number of resistance mechanisms such as efflux pump, genetic adaptation, enzyme-linked mediated resistance, outer membrane structure as well as difference in genetic makeup and mutation (Prescott, 2005; Patrick *et al.*, 1991).

The Sensitivity of the fungus, *Aspergillus* sp. to the toxicity of the different toxicants (Mymy and Zip)with the two different water samples decreased in the following order (noting the lower the lethal concentration(Lc_{50}), the more toxic the toxicant); Zip detergent in freshwater (37.26mg/l) >Mymy detergent in brackish water (41.71mg/l) >Zip detergent in brackish water (42.63mg/l) >Mymy detergent in fresh water (47.82mg/l).

As analyzed from the median lethal concentration (Lc_{50}) of the toxicants, the study showed that the toxicity of the detergent is to a greater extent affected by the salinity of the medium. Zip detergent was more toxic in fresh water with Lc_{50} of 37.26mg/land less toxic in brackish water with Lc_{50} of 42.63mg/l. This may be as a result of the chemical reactions of some components of the detergent with the salinity content of the surrounding medium. However, Mymy detergent in fresh water showed less toxicity with Lc_{50} of 47.82mg/land appeared to be more toxic in brackish water with Lc_{50} of 41.71mg/l as seen in table 5 above.

5.2 Conclusion

The study revealed that the fungal species; *Aspergillus niger* responded differently to different concentrations of the two different toxicants with respect to time. The response of the organism due to the presence of the toxicant may alter or affect the process of decomposition, mineralization and nutrient generation which will in turn disturb the nature of the ecosystem and biogeochemical cycle. Furthermore, the study also revealed that domestic detergents contain certain synthetic chemicals that could be very hazardous to life reducing productivity and promoting death of not just the aquatic forms of life (both the micro and macro flora) but also higher forms of life such as humans and other mammals that make use of them as source of food and other means of survival. The production industries should make use of contemporary methods such as recovering and recycling of the toxic components of detergent and the use of biosurfactants for biodegradation during the production of detergent. The concentration of the synthetic chemicals used in the production of detergents should be reduced.

References

American Public Health Association (APHA)(1992). Standard Methods for the Examination of Water and Waste Water,18th edition. APHA, Washington D.C.,USA.

American Public Health Association (APHA)(1998). Standard Methods for the Examination of Water and Waste Water,17th edition. APHA, Washington D.C.,USA,1391.

Akponah, E; Otutu, J. O; Akpomie, O. O; Ubogu M. (2014). Evaluation of the acute toxicity of three locally synthesized dyes (3,5-dimethoxypyridine azo-6-methyl uracil, 5-ethoxybenjothiazole azobenzoloxyphenol and 4-ethyl 5,2,3-thiazole azo-6- methyl uracil) using *Nitrosomonas* sp. and *Nitrobacter* sp. *The Asian Journal of Applied Microbiology***2**: 18-25

Atuanyan E. and Tudararo-Aherobo L. (2015). Ecotoxicological effects of discharge of Nigerian petroleum refinery oily sludge on biological sentinels. *Journal Environmental Science and Technology*. **9 (2)**: 95-103

Birkett, J.W., Lester, J.N. (2003) Endocrine disrupters in wastewater and sludge treatment processes. *Lewis ublishers*, Boca Raton.

Contesini, F.J., Lopes, D.B., Macedo, G.A., da Graca Nascimento. M., de Oliveira Carvalho P. (2010) Aspergillus sp. Lipase: Potential biocatalyst for industrial use. *Journal of Molecular Catalysis B: Enzymatic*,**67**:163-171

Clark R. B. (1992). *Marine pollution*3rd edition, Gook raft Great Britain, UK, 50-60.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries*, part 2, 2nd edition, Cambridge University press. New York.

de Vries, R. P. (2003). Regulation of Aspergillusgenes encoding plant cell wall polysac-charide-degrading enzymes; relevance for industrial production. *Appl. Microbiol.Biotechnol.***61**:10–20.

Fawole O.B., Odunfa S.A. (2003) Some factors affecting production of pectic enzymes by *Aspergillus niger. International Biodeterioration and Biodegradation*, **52**:223-227.

Focht, I. M. and Weslake, D. W. S. (1987). Biodegradation of hydrocarbon in fresh water In: J. H. Vandermenden and S. E. Honday (ed). Oil in freshwater. *Chemistry and biology. Countermeasure Technology*. Pergemon Press, New York.

León V.M., López C., Lara-Martín P.A., Prats D., Varó P., González-Mazo E. (2006) Removal of linear alkylbenzenesulfonates and their degradation intermediates at low temperatures during activated sludge treatment. *Chemosphere*, **64**: 1157-1166.

Lenntech (2008). Toxicity of chemicals to aquatic species. Lenntech Water Treatmentand Air Purification Holding B. V. *J. Aquatic* Rotterdamseweg 402 M, 2629HH Delft, The Netherlands. 1-2.

Moreno, G., Lubian, L.M., and Soarest, A.M.V.M. (2000). Influence of cellular density on determination of EC50 in microalgal growth inhibition tests. *Ecotoxicology and Environmental Safety*. **47**: 112 – 116.

Obire, O. and Nrior, R. R. (2014). Effect of concentrate detergents on *Pseudomonas fluorescens* and *Mucor racemosus*. *Journal of International Society of Comparative Education, Science and Technology*, 1: 189-199.

Odokuma L. O. and Akponah E. (2008). Response of *Nitrosomonas, Nitrobacter* and *Escherichiacoli* to drilling fluids. *Journal of Cell and Animal Biology*, **2 (2)**: 043-054

Odokuma L. O. and Okpokwasili G. C. (2003). Bacterial enzyme biosynthesis inhibition: A

tool for ecotoxicity assay. *Glob. J. Pur and Appl Sci.***9**: 311-318.

Patrick, J.E., Mang, D.T. and Young, L.T. (1991). Degradation of toluene and m-xxylene and transformation of o-xylene by denitrifying enrichment cultures. *Applied Environmental Microbiology.***57**: 450-454.

Stojanović, J., Jakovljević, V.,Matović, I., Gajović, O.,Mijušković, Z. and Nedeljković, T. (2011) Influence of detergent on metabolic activity of fungi, *Aspergillus niger*, *Natural Science.***3**: 466-470

Williams, J. O. and Dimbu, P. C. (2015), Effect of abattoir waste water on soil microbial communities. *Scholars Academic Journal of Bioscience.***3 (5)**: 452-455

Williams J.O, Money J. (2017) Assessment of the microbiological and physicochemical quality of two Rivers receiving in organic fertilizer inputs in Ogoniland, Rivers State, Nigeria. *Journal of Environmental Science, Toxicology and Food Technology.***2(1)**:48- 52.

Williams, J.O., Madise, E.(2018) Physicochemical and Microbiological quality of a creek in the Niger Delta region of Nigeria. *Journal of Advances in Microbiology*, **10(1)**:1-10

Williamson, K. J. and Johnson, O. G. (1981). A bacterial bioassay for assessment of waste water toxicity. *Water Research*, **15**: 383- 390.

Zheng, C., Riccardo, B and Guglielmina, F. (2003). EC50 estimation of antioxidant activity in DPPH assay using several statistical programs. *Food Chemistry*, **138**: 414 – 420